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Evaluation of Intact Cell Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry for Capillary Electrophoresis Detection of Controlled Bacterial Clumping

Pomastowski P[1,2], Szultka-Młyńska M[1,2], Kupczyk W[3,2], Jackowski M[3,2] and Buszewski B[1,2*]

[1]*Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Poland*
[2]*Interdisciplinary Centre of Modern Technology, Nicolaus Copernicus University, Poland*
[3]*Department of Surgery, Collegium Medicum, Nicolaus Copernicus University, Poland*

Abstract

Matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been proposed as a technique of choice for quick classification of bacterial cells and precise characterization of microorganisms. The evaluated MALDI MS procedure was confronted with automated biochemical method using the VITEK 2 Compact system employed as a reference method. Moreover, the investigation aimed at describing and optimization of the environmental conditions having an impact on reproducibility and quality of one-dimensional intact-cell MALDI TOF spectra. The work presents off-line combination of capillary electrophoresis of microbial clumping with spectrometric detection.

Keywords: Identification of bacteria; Matrix-assisted laser desorption/Ionization mass spectrometry; Intact cell analysis; Capillary electrophoresis; Clumping

Introduction

One of the fundamental stages of microbial analysis is identification and characterization of microorganisms. In modern medicine early detection of pathogens provides an opportunity to determine the risk of neoplastic process in the infected organs, and to implement appropriate preventive and screening actions, which would minimize the risk of developing the disease [1]. In the case of food (e.g., dairy) and pharmaceutical industry, identification of microorganisms is an essential step of a technological process, which determines the quality of the manufactured product [2]. Furthermore, in medicine - detection of a disease in an early stage of development would allow to achieve better results of a treatment, in industry - identification of pathogens in early stages of a technological process saves money, production time and influences improvements in the decision-making processes [3]. Classical methods for identification of microorganisms commonly used in routine microbiological laboratories are based on a set of tests and analyses of biochemical properties of microorganisms, as well as on methods utilizing antigen-antibody reactions [4]. Recent years have brought increasing interest in automation of identification methods based on a study of biochemical characteristics of microorganisms, along with improvement of serological methods, causing a significant increase in the diversity of rapid identification tests [5]. Nevertheless, waiting for the result of identification is still long enough, and depending on the method and the analytical system, it ranges from 4-8 hours for automated methods to about 24 hours for semi-automatic ones [4,5]. Therefore, emphasis has been put on the search for new, precise and rapid methods of identification of microorganisms.

One of them is the use of capillary electrophoresis approaches for analysis of bacterial and yeast cells [6]. Unfortunately, this technique bases on conventional and restricted UV- or diode array detection (DAD) and requires long time for optimization of separation process [6,7]. Solving the problem of detection, raising selectivity and reducing the time to identify microorganisms is possible with the use of intact cell matrix-assisted laser-desorption/ionization (IC MALDI) time-of-flight mass spectrometry (TOF MS) method [1-5,8]. Spectrometric analysis of a sample with this method provides comprehensive information within a couple of minutes. In addition, one of the advantages of IC MALDI method is the fact that only one (single) colony is needed for microbiological analysis [1,8]. Moreover, some released protocols enable direct IC MALDI analysis of bacteria cells from infected blood [8] or milk [9]. This procedure is based on analysis of the unique protein profile of a microorganism, known as molecular fingerprint (MF) [1,9]. In this method, a pure culture of a microorganism is applied as a thin layer on a MALDI target. Then, solution of a matrix is deposited, mainly HCCA (α-cyano-4-hydroxycinnamic acid), DHB (dihydroxybenzoic acid), SA (synapic acid) dissolved in an organic solvent.

The matrix serves as a medium for extraction and crystallization of proteins (and other components such as lipopolysaccharides), mainly bacterial ribosomal proteins, which are present in the largest amount in microorganism cells [9,10]. During evaporation of the solvent the extracted bacterial proteins undergo co-crystallization with the matrix and thus formed crystals are subsequently subjected to pulsed laser beam irradiation. During this process the matrix crystals absorb high-energy photons of a specific wavelength which results in desorption and ionization of the matrix and desorption of protein molecules. In high vacuum of the ionization chamber the matrix protons collide with neutral molecules of proteins giving them a positive charge, so that protein and peptide molecules get ionized [1,11]. Positive ions of the matrix and the proteins are then accelerated in electrostatic field and move towards electrodes whose special design allows passage

***Corresponding author:** Buszewski B, Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Gagarina 7, 87-100 Torun, Poland, Tel: +48566114308; Fax: +48566114837; E-mail: bbusz@chem.umk.pl

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through the electrode. The rate of passage of ions through individual electrodes is inversely proportional to their mass and charge. After passing through the electrode, the ions migrate towards the detector, freely moving through the region, in which electrostatic field no longer operates (field-free drift region). The differences in molecular mass and charge cause that each molecule requires different time to reach the detector. The detector records the time of flight of ions and automatically generates a spectrum mainly corresponding to protein and peptide ions of different mass to charge ratio. The resulting spectrum of the tested strain is then compared with reference spectra of microorganisms, and automatically assigned to give identification of the organism [12,13].

Four commercial systems are mainly used in routine identification of microorganism, namely: BioTyper, SARAMIS, VITEK MS and Andromas, provided by Bruker, Shimadzu, bioMérieux and Andromas SAS, respectively [14-17]. Unfortunately, the cost of the software and the required licenses are often a limiting factor for a common use of these systems. Therefore, a homemade database (repository) can be developed and applied to targeted identification of microorganisms and as a complementary method to classical molecular or biochemical techniques. Moreover, a local repository of reference bacterial strains can also be used for statistic evaluation of one-dimensional IC MALDI spectra [18] or in a detection approach for separation analysis of microorganisms.

The aim of this study was evaluation of conditions influencing reproducibly and quality of bacterial spectra. Furthermore, the acquired spectra were to serve as a basis for a homemade repository. The investigation was to verify whether implementation of one-dimensional MALDI TOF MS system could be successful in identification and characterization of selected bacterial cells, also in the case of coupling of IC MALDI technique with capillary electrophoresis.

Materials and Methods

Chemicals

Acetonitrile (ACN), ethanol, trifluoroacetic acid (TFA), hydrochloric acid, sodium hydroxide solution, TRIS (tris(hydroxymethyl)aminomethane), boric acid (B) and calcium nitrate were purchased from Sigma Aldrich (Steinheim, Germany). Ultra-pure water from a Milli-Q water system (Millipore, Bedford, MS, USA) was used throughout the work.

All chemicals for MALDI-MS analyses were supplied at the highest commercially available purity from Fluka Feinchemikalien GmbH (part of Sigma Aldrich). Ground steel targets (Bruker Daltonik, Bremen, Germany) were used for sample deposition. α-cyano-4-hydroxycinnamic acid (HCCA), 2,5-dihydroxybenzoic acid (DHB) and sinapinic acid (SA) were employed as matrices for MALDI analysis of intact bacterial cells (dried droplet method), Bruker Bacterial Test Standard (BTS) were used for external calibration.

Culture media

Agar plates for bacterial growth were obtained from Pol-Aura, Dywity, Poland (Soybean Casein Digest Agar (SCDA), R2A Agar (R2A), Mueller Hinton (MH2), chocolate agar (CA), Schaedler Broth (SB)) and from bioMérieux, Warsaw Poland (Columbia CNA Agar supplemented with Colistin-Nalidixic Acid (COS), MacConkey Agar (MCK), Schaedler agar with 5% sheep blood (SCHE), SCDA).

Bacteria culture: The bacterial strains were used during investigations: *Arthrobacter psychrolactophilus* ATCC 700733, *Klebsiella pneumoniae* ATCC700603, *Bacillus cereus* ATCC 10876 and ATCC 13061, *Bacillus subtilis* ATCC 19659 and ATCC 11774, *Escherichia coli* ATCC 25922, ATCC 10536, *Lactococcus lactis subsp. lactis* ATCC 11454 and ATCC 19435, *Micrococcus luteus* ATCC 10240, *Pseudomonas putida* ATCC 31483 and *Pseudomonas aeruginosa* ATCC 27853 were obtained from Pol-Aura. Another strain of *Lactococcus lactis subsp. lactis* was obtained from the collection of the Department of Microbiology (Nicolaus Copernicus University, Torun, Poland).

Initially, the analyzed bacterial strains were cultivated at 37°C for 17 h on different solid (SCDA, R2A, MH2, CA, MCK, COS, SCHE) and liquid media (SB, TSB) to examine how different culture media influences the obtained MS spectra. In the next step, bacterial strains were cultivated at 37°C and seven different times of incubation: 12 h, 17 h, 24 h, 36 h, 48 h, 72 h and 96 h to control the effect of time on the composition and quality ICM MS spectra. After each incubation period increment of CFU (colony forming unit) was determined and additionally OD_{600} (optical density measured spectrophotometrically at 600 nm) was examined in the case of liquid media. Bacterial colonies were identified using Vitek 2 Compact system, according to the manufacturer's instructions, with identification cards GP (Gram positive bacterial identification), GN (Gram negative bacterial identification) and ANC (anaerobic bacteria and coryneform bacteria identification) taking into account cultural, morphological and biochemical criteria.

Spectrometric analysis of bacteria: Initially, bacterial colonies were smeared on a MALDI target as a thin layer and overlaid with 1.0 µl of matrix solution: DHB (50 mg/mL), HCCA (10 mg/mL) or SA (20 mg/mL) in a solvent mixture (EtOH/ACN/H_2O, 1:1:1 (v/v/v) to examine the impact of the kind of a matrix on recorded spectra [16]. The TFA solution was added to solvent mixture to 0.1-2.5% v/v final concentrations. Since the obtained results were not satisfactory, the part of the protocol was modified. Bacterial samples were transferred from agar plate to the above-mentioned acidified solvent mixture so that OD_{600} of the final bacterial suspension ranged between 0.04-0.6 (10^9-10^{12} cells per mL). In order to evaluate how this change affects the amount of cells per spot, the bacterial suspension was diluted with the solvent mixture at 1:1, 1:10, 1:100, 1:1000 and 1:10 000.

Intact cell mass spectrometric analysis was conducted with the use of ultrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with a modified Nd:YAG laser (smartbeam II™) operating at the wavelength of 355 nm and the frequency of 2 kHz. ICM MS spectra of were recorded in linear positive mode within *m/z* range of 300-30 000 and applying the acceleration voltage of 25 kV. All mass spectra were acquired and processed with the dedicated software: flexControl and flexAnalysis, respectively (both from Bruker). Cluster analysis with Ward's method and Euclidean distances of the obtained ICM MS spectra, as well as verification of the indicated characteristic peaks was performed with the use of Statistica software (StatSoft, Krakow, Poland).

Electrophoresis of microbial clumping

Bacterial cells were rinsed with water twice, then suspended in 0.005M $Ca(NO_3)_2$ solution in order to modify the surface charges, and after 1 h the pellet with bacterial cells was washed again to remove free Ca^{2+}_{aq} ions [19]. The final bacterial sample was suspended in an outlet buffer. OD_{600} of the bacterial suspension was estimated between 0.04-0.6.

Capillary electrophoresis (CE) experiments were performed with

an HP^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with DAD and fused silica capillaries (id=75 μm; L_{tot}=33.5 cm; L_{eff}=25 cm; Composite Metal Services, Shipley, UK). New capillaries were rinsed before use with 1.0M NaOH, deionized water, and BGE (background electrophoretic buffer) for 10 min each. The electrophoretic analysis was performed in a nonlinear system, namely: outlet buffer: TB (C_{TRIS}=4.5 mM, C_B=50 mM, pH 8.3), inlet buffer: TB-HCl (C_{TRIS}=4.5 mM, C_B=50 mM, C_{HCl}=4.4 pH 7.15), I_{max}=100 μA, U=15 kV, t=23°C, λ=214 nm, and injection in the pressure mode at 50 mbar for 25 s. Between runs, the capillaries were washed with 1.0M NaOH, deionized water - for 2 min each, and a running buffer for 4 min. A total volume of 0.5 mL stock bacterial suspension was used for electrophoretic measurements [19]. The focused fractions of bacterial clumping were collected in CE-MS mode, diluted and transferred to a MALDI target according to the above-mentioned procedure.

Results and Discussion

Spectrometric evaluation of environmental conditions

Initially, all 13 bacterial strains were cultured on various solid agar plates and two kinds of liquid media, at 37°C for 17 h in aerobic conditions to demonstrate the impact of different media (dedicated or not to culture examined microbial strain) on quality of IC MALDI spectra of cultured bacterial cell. It was observed that the best growth was reached in the case of Schaedler media, both agar and liquid, for all the examined microbial strains. Although Schaedler media are intended for cultivation of obligate anaerobe or strict anaerobe, the examined bacteria also exhibited stable, reproducible and fast growth. After optimization of the growth conditions the bacterial species were cultured for different time intervals: 12 h, 17 h, 24 h, 36 h, 48 h, 72 h and 96 h, in solid and liquid medium. After that, increment of CFU and OD_{600} was measured for all bacteria species which were cultured on SCDA and SCHE media (Figure 1B). The examined bacterial strains exhibited three stages of growth. The first phase, with the incubation time between 0-24 ± 12 h, was the growth stage - microbial cells were viable and growing. The second stage (24-36 ± 12 h) was the stationary (plateau) phase, and the last one was the death phase (48-96 h). For the last two stages the cells were non-growing and viable, and non-growing, non-viable, respectively [20]. IC MALDI spectra were registered for all bacterial colonies incubated in various incubation time intervals (Figure 1A).

It was shown for all bacterial species that various incubation time intervals influenced quality of the registered spectra. No major qualitative changes in peak patterns were detected, however increase of signal intensity over time (100% per 24 h) and drift of baseline were observed. This results directly from the current stage of microbial growth [1,2,21]. In the plateau and the death phase degradation of some enzyme complexes is initiated, and in the case of e.g., *Bacillus* species in the plateau phase, a spore is formed [1,20]. Qualitative hanges in the composition of cells translates directly into the quality of the registered spectrum. The obtained results are in accordance with previous studies [1,2,21]. In the case of the culture of *E. coli* or *S. aureus* it is possible to register reproducible and good quality spectra between the time intervals of 5-48 h [21]. On the other hand, in routine IC MALDI identification of microorganisms it is necessary to keep the time of bacterial cell incubation the same as in the case of reference bacteria. Otherwise, it may result in misidentification of bacterial species [1-7,14-17].

Initially, spectra were recorded for bacterial colonies coming from agar plates and liquid media deposited directly on a MALDI target.

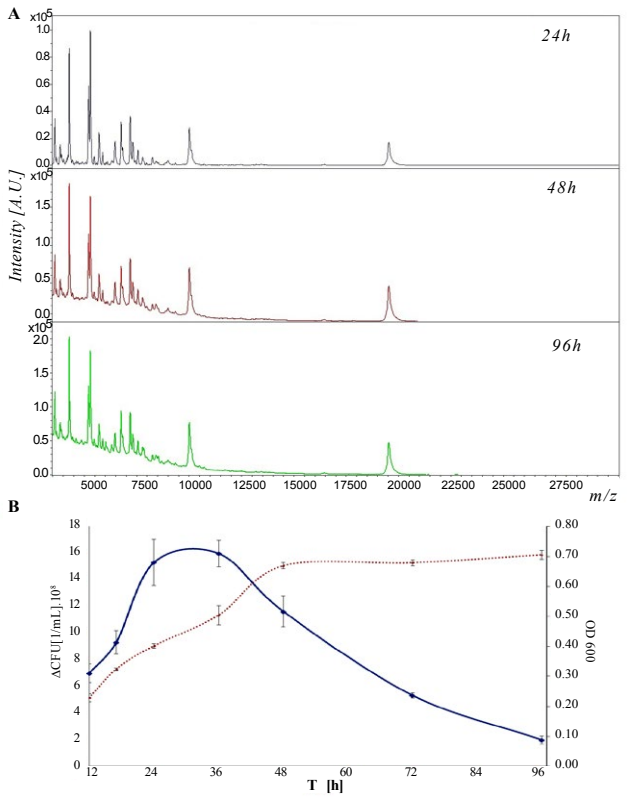

Figure 1: MALDI-TOF MS spectra of B. cereus ATCC 10876 cultured on SCHE incubated in different time intervals (A) and its growth kinetics (Dotted line: OD600, Stroked line: Increment of CFU) (B).

It was observed that IC MALDI spectra of the bacteria cultured in liquid media exhibited better quality, reproducibility and intensity. The spectra registered for smeared colonies from diverse agar plates were relatively irreproducible and many peaks originating from media themselves were observed. In order to improve the reproducibility and to eliminate the medium effect, each bacterial colony was placed in a solvent mixture and then onto a MALDI target. The obtained spectra were more intensive, reproducible and the signals coming from culture media were eliminated. Subsequently, influence of culture media on the quality of the obtained IC MALDI spectra was tested (Figure 2A).

It was shown that MALDI spectra of bacteria cultured on different agar plates were mutually consistent and only few peaks altered in intensity or were missing. Subsequently, the number of cells per spot was optimized (Figure 2B). This criterion is crucial in evaluation of IC MALDI spectra [1,2]. The optimal OD_{600} value of all analyzed bacterial suspensions was in the range of 0.80-0.04. These values represent 10^5-10^9 cells per spot. Too small amount of microbial cells (less than 10^4) is insufficient to generate a spectrum, while in the case of excessive amount of cells (greater than 10^{10}), it is impossible to reach desorption and ionization of bacterial components, and hence to register a spectrum [2,22]. It has been shown that one loopful of bacterial cells per spot, or 1 mL of suspended solution (e.g., *E. coli*, *E. faecalis*), is sufficient for registering good quality spectra [1,8]. In the case of IC MALDI analysis of bacteria cell taken directly from a biological sample (blood, milk) the minimal value is 10^3-10^5 cells per μL [8,9].

In this investigation IC MALDI spectra were registered using

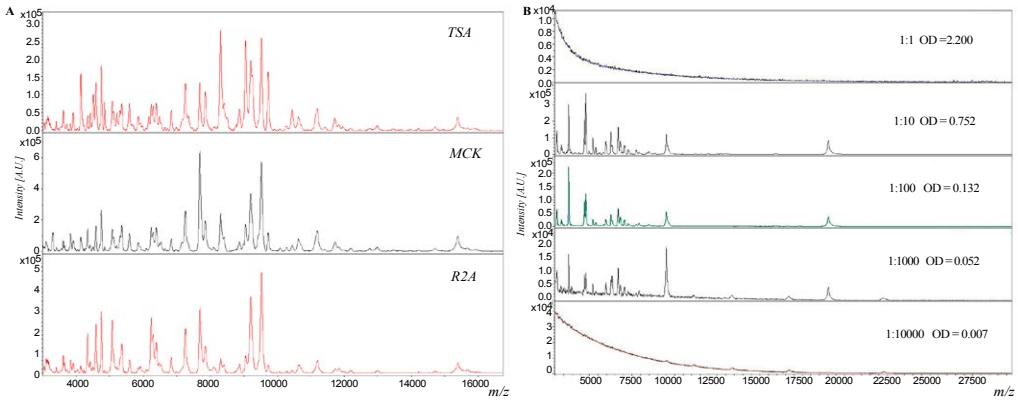

Figure 2: IC MALDI spectra of bacteria cultured on different culture media (A), and MALDI-TOF MS spectra of B. cereus ATCC 13061 with 17 h incubation time, Schaedler agar, HCCA as the matrix, registered for various dilution factors and OD600 values (B).

various matrices, namely: SA, HCCA and DHB. The worst results were obtained for sinapinic acid. Application of HCCA and DHB gave reproducible spectra with high resolution and stable ionization (Figure 3A).

In screening analysis of microbial cells the use of these two matrices is common [23-32]. However, the spectra recorded with different matrices had different distribution of peaks (Figure 3B, 3C). Spectra recorded using DHB as a matrix (Figure 3B) were richer in signals in the case of analysis of *Bacillus sp.* and *L. latcis*. Worse results were reported for HCCA spectra (Figure 3A) of Gram-positive bacteria. Peptidoglycan is the main component of cell walls of these bacteria. Probably disruption of cell walls during the laser ablation and ionization process was hindered by the multi-layer nature of peptidoglycan [14]. Therefore, it was impossible to ionize a sufficient number of proteins. DHB matrix has been reported to facilitate cell wall disruption and protein extraction [1,14,27-32].

The presented dendrograms exhibit different relations between various IC MALDI spectra registered using various matrix (Figure 3B, 3C). In the case DHB-based spectra the highest similarity was reported for *A. psychrolactophilus* and *E. coli*, *M. luteus* and *P. aeruginosa*, *B. cereus* and *L. lacis*. However, in the case of cluster analysis of HCCA-based spectra, the highest similarity was observed for *A. psychrolactophilus* and *P. putida*, *M. luteus* and *P. aeruginosa*. Nevertheless, HCCA spectra exhibited more significant differences between similar clusters. This may result from different nature of the two matrices [1]. DHB is characterized with large needles (Figure 3B), whereas HCCA forms fine crystals (Figure 3C). The different nature of crystallization process results in different extraction mechanism of bacterial components. The signals obtained for ICM measurement were mostly of ribosomal-protein origin [25-28]. Circa 50% of the peaks of all bacteria unambiguously represented basic ribosomal proteins [1]. The rest are cold-shock proteins, DNA-binding proteins and outer-membrane lipoproteins [14]. Therefore, DHB will be the most favorable matrix for the analysis of lipoproteins, whereas in the case of intact cell measurements of DNA-binding proteins the use of HCCA will result in a higher number of signals [14,20,25-29]. The similarities of spectra are not correlated in genetic genetic origin of phylogenetic trees.

Identification of selected bacterial cells

In this study 13 different bacterial species, 510 various independent biological samples (8 replicates for each sample) were examined. All IC MALDI results of the tested bacterial stains were confirmed directly by classical standard microbiological testing using biochemical identification system (Vitek, bioMérieux) (Table 1).

Reference spectra were registered for all bacterial species. Then, the species were randomly selected for identification based on comparison of characteristic peak patterns and characteristic signal. The characteristic peaks were indicated using U-Mann-Whitney test (UMW) - one of the nonparametric test for independent samples. It is often used by researchers for statistical evaluation of spectrometric data [22]. The experimental data did not meet the assumptions of the Student's t test and therefore this test could not be applied. Characteristic peaks were identified via independent comparison within a group. Table 1 summarizes the values of *m/z* which met the criteria of UMW test.

The obtained results of spectrometric identification of microbial species were in agreement with the ones of the biochemical method. Nevertheless, the spectrometric procedure is faster, more precise and efficient. In the case of biochemical analysis, the time and the use of expensive identification cards were the limiting factors. Moreover, spectrometric analysis was performed in a larger number of repetitions, due to lower cost of a single analysis, shorter time and easiness of preparation.

Capillary electrophoresis of microbial clumping with IC MALDI detection

According to the electrokinetic theory, the charge on a surface, determined by the characteristic properties of the membrane components (e.g., proteins, lipopolysaccharides), influences the behavior of microorganisms in the electric field [7]. This phenomenon determines electrophoretic mobility, which enables separation of bacteria cells (biocolloids) [6]. However, analysis of such complex systems is related to a number of problems, such as uncontrolled clumping (aggregation) and/or adhesion to the inner surface of a capillary. Another problem is detection of microbes. Common UV and DAD system do not allow for obtaining specific spectra that would enable a satisfactory identification of separated bacterial cells. The obtained spectra do not provide sufficient information about the aggregation of microorganisms [7]. Moreover, conventional linear buffer system is not effective in this case.

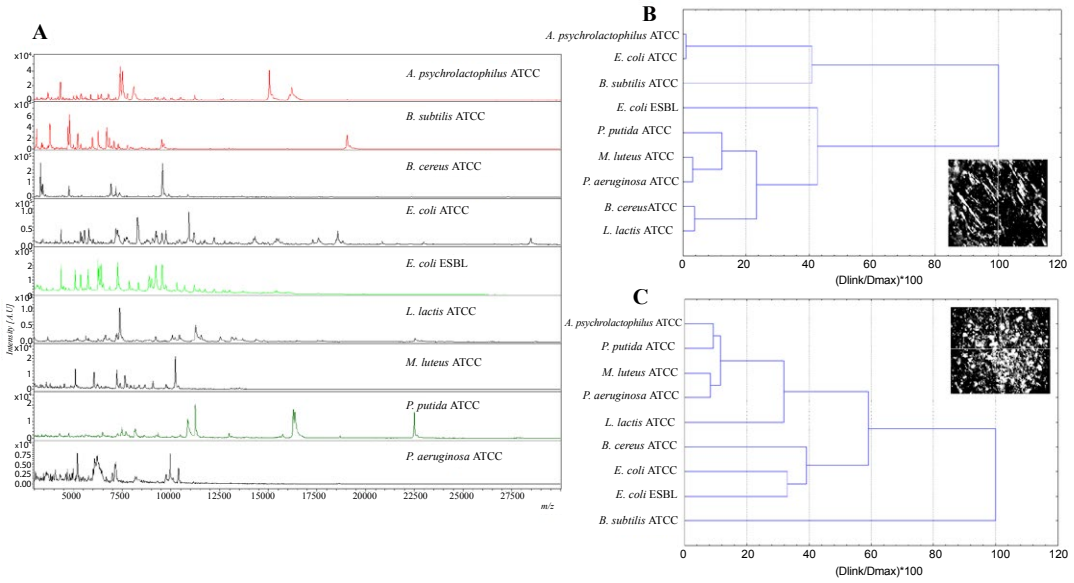

Figure 3: Intact cell MALDI-TOF MS spectra of the analyzed bacteria strains registered using HCCA matrix (A); dendrograms for DHB- (B) and HCCA-spectra (C).

Bacterial strain	VITEK 2			IC-MALDI TOF MS				CE	
	n	Match (P,%)	Mismatch	n	Match (P,%)	Mismatch	Characteristic signals ± SD (m/z)	n	T ± SD (min)
Arthrobacter psychrolactophilus ATCC 700733	5	4(80)	1	40	39(98)	1	7393 ± 2, 15056 ± 3, 16202 ± 3	10	1.88 ± 0.09
Bacillus cereus ATCC 10876 *Bacillus cereus* ATCC 13061	4 4	4(100) 4(100)	0 0	50 40	50(100) 40 (100)	0 0	4774 ± 2, 6939 ± 3, 9563 ± 3, 13630 ± 2	10	2.20 ± 0.03
Bacillus subtilis ATCC 19659 *Bacillus subtilis* ATCC 11774	4 4	4(100) 4(100)	0 0	50 50	50(100) 50(100)	0 0	3799 ± 3, 9532 ± 3, 19070 ± 5, 19734 ± 1	10	2.02 ± 0.04
Escherichia coli ATCC 25922 *Escherichia coli* ATCC 10536	5 5	5(100) 4(80)	0 0	30 30	30(100) 30(100)	0 0	3323 ± 2, 7695 ± 7, 10451 ± 7, 18576 ± 6, 20867 ± 2,	10	3.21 ± 0.05
Klebsiella pneumoniae ATCC700603	5	5(100)	0	40	40(100)	0	4363 ± 2, 6258 ± 4, 9545 ± 3	10	3.56 ± 0.06
Lactococcus lactis subsp. lactis ATCC 11454 *Lactococcus lactis subsp. lactis* ATCC 19435	3 3	2(67) 3(100)	0 0	30 30	30(100) 30(100)	0 0	6632 ± 4, 7352 ± 6, 11256 ± 5, 22550 ± 2	10	2.36 ± 0.03
Micrococcus luteus ATCC 10240	3	3(100)	0	30	30(100)	0	5104 ± 2, 7237 ± 4, 10225 ± 4	10	2.45 ± 0.04
Pseudomonas putida ATCC 31483	3	2(67)	0	30	30(100)	0	16284 ± 6, 22511 ± 3, 10485 ± 2	10	3.74 ± 0.03
Pseudomonas aeruginosa - ATCC 27853	3	3(100)	0	30	30(100)	0	5203 ± 3, 7173 ± 5, 9967 ± 6	10	3.69 ± 0.07

n-number of identification; P-percentage of success identification (mach/n*100%), t-electromigration time

Table 1: Identification of selected bacterial cells using biochemical and ICM MS methods.

In the presented study a specially designed homemade databases of intact cell spectra were used for development of a method of capillary electrophoresis separation of microbial clumping coupled with IC MALDI identification. Figure 4 presents the example electropherograms and spectra of *L. lactis* and *E. coli.*

The spectra of reference species were in agreement with the ones obtained after electrophoretic separation. The characteristic spectrometrics peak of the focused bacterial cells was consistent with the reference values. The observed small increase and decrease of the signal intensity and modification of some signals, result from sorption of calcium ions onto the proteins [14,33]. Modification of bacteria with calcium ions determines the change in their electrophoretic mobility and reduces repulsive forces. This results in creation of controlled clumping and signal amplification [19]. Application of buffers with different ions mobilities (an isotachophoretic mode) and without bacterial surface modification with Ca^{2+} allowed focusing the zones [19]. Therefore, it was possible to collect focused microbial cells and to detect their IC MALDI spectra [34]. The electrophoretic analysis was reproducible, as evidenced by the low standard deviation of electromigration time (Table 1). Moreover, it was shown the higher electromigration time of analyzed Gram-negative in comparison with Gram-positive bacterial cells (Table 1). It probably results from higher zeta potential value of Gram-positive bacteria in used buffer system and as a consequences weaker interaction with deprotonated silanol groups [7]. The performed investigation proves that application of CE-

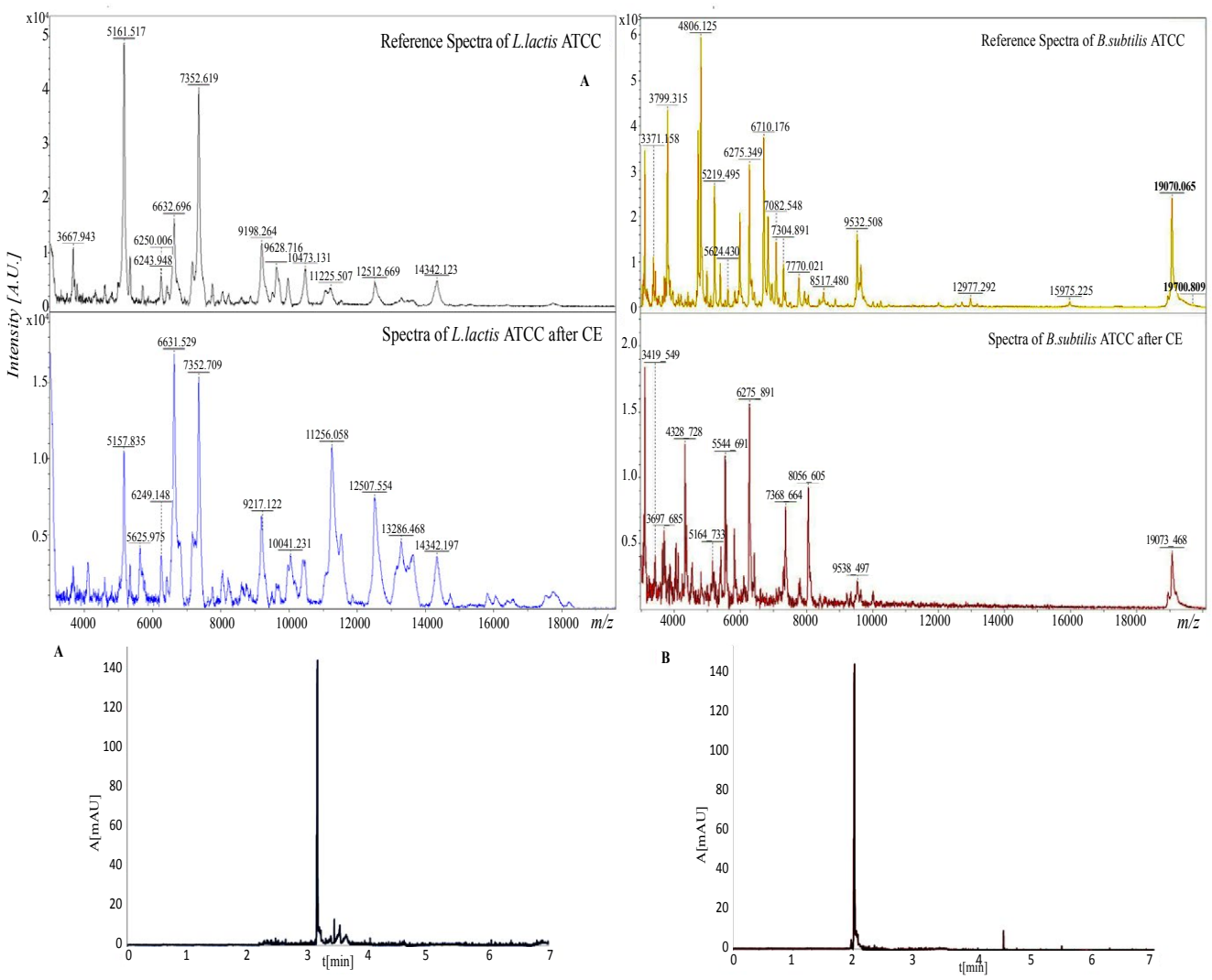

Figure 4: Electropherograms of *L. lactis* ATCC 19435 (A) and *E. coli* ATCC 25922 (B) along with MALDI TOF MS spectra used for identification.

MALDI TOF MS approach may be useful in quantitative analysis of controlled clumping in routine bioanalysis of bacterial cells.

Conclusion

One-dimensional IC MALDI is has recently found application as a fast and alternative method of microbial identification to classical biochemical methods. An important advantage of ICM MS is very short time of analysis and the possibility of full identification of the microorganisms of interest. However, this strategy requires thorough evaluation of environmental conditions having strong impact on reproducibly of fingerprint spectra. Repeatability of ICM MS spectra largely depends on the type of the matrix, the incubation temperature and the amount of cells per spot. The kind of a culture medium does not influence significantly the quality of spectra. Apart from signal matching, searching for specific biomarkers of a given species is an effective method of microbial identification. MALDI TOF MS has proven to be a promising detection system for capillary electrophoresis of bacterial clumping.

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